Expert Opinion

Methotrexate: a detailed review on drug delivery and clinical aspects

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Introduction: Uses of methotrexate (MTX) are well established for the treatment of various types of malignancy, psoriasis, rheumatological diseases and the medical termination of pregnancy. Formulation and targeting approaches for MTX with controlled release carriers, multiparticulate systems, prodrug and drug conjugates have been found to improve bioavailability, reduce adverse effects and maximize clinical efficacy, compared with conventional methods.

Areas covered: This exhaustive literature survey on different electronic databases covers drug delivery and clinical trials on MTX. This review deals with the challenges and achievements of controlled release, multiparticulate, prodrug and drug conjugate systems of MTX.

Expert opinion: Therapeutic drug monitoring of MTX is crucial to attain a good efficacy. In spite of the advantages of multiparticulate, prodrug and drug conjugates, clinical applications of such formulations of MTX are still under infancy. These drug delivery systems require the special attention of medical experts for its wider clinical usage, and pharmaceutical experts for its scale-up. The combination of MTX with other antineoplastic and immunosuppressants should also be subjected to clinical trials, such as the combination of misoprostol with MTX in abortion.

Keywords: abortion, cancer, liposomes, methotrexate, misoprostol, nanoparticles, prodrug and drug conjugates, rheumatoid arthritis

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1. Introduction

The ultimate goal of pharmaceutical scientist is to maximize therapeutic efficacy and minimize possible hazards of drug(s). Chemotherapeutic agent needs special attention of novel drug delivery approaches due to their need at target site. Though conventional dosage forms are simple and economic, they are not as effective as site-specific drug delivery systems in terms of maximum therapeutic outcomes with lesser side effects. Though a bit of expensive and high risk of failure during scale-up, the novel drug delivery systems possess to achieve the ultimate goal of efficacious delivery. Methotrexate (MTX) is clinically used for the treatment of cancer, autoimmune diseases and induction of abortion with misoprostol.

Sidney Farber and coworkers stated that aminopterin, a folic acid analog discovered (1947) by Yellapragada Subbarao, could cure acute lymphoblastic leukemia in children. The development of folic acid analogs was continued based on the fact that folic acid supply could worsen the leukemia and deficiency of folic acid improve the curative action of folic acid analogs. By 1950, MTX (formerly introduced as *amethopterin*) replaced aminopterin, the most toxic antitumor treatment for leukemia [1]. The first results of preclinical and clinical studies

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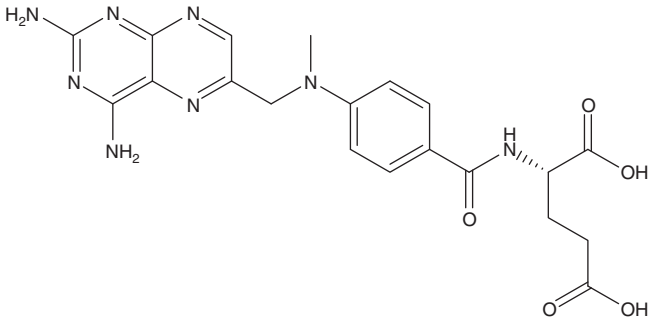
Box 1. Drug summary.

Drug name	Methotrexate
Phase	Phase I, Phase II
Indication	Cancer, abortion, rheumatoid arthritis, psoriasis and other autoimmune diseases
Mechanism of action	Inhibition of dihydro folate reductase (DHFR), interleukins (IL-1, IL-6, tumor necrosis factor 1 (TNF) activity and other inflammatory cytokines
Chemical structure	

published in 1956 showed that the therapeutic margin of MTX was better than that of aminopterin. In the same year, efficacy of MTX in choriocarcinoma was established [2]. This drug was further explored for many other cancers either alone or in combination with many other drugs, and extensively studied for other noncancer indications in the 1970s. In 1988 and 2002, this drug has been approved by US Food and Drug Administration (FDA) for the treatment of rheumatoid arthritis (RA) and Crohn's disease, respectively [3,4].

Fixed-dose combination of MTX and misoprostol is found to be best for abortion than individual doses. Therefore, present review deals with the achievements and challenges of MTX (a chemotherapeutic agent), its drug delivery systems from conventional to novel approaches along with its clinical aspects.

1.1 Pharmacokinetics

MTX, a Biopharmaceutical Classification Systems class III drug, is a weak dicarboxylic acid having pKa value 4.7 – 5.5. MTX possess low permeability (C logP = 0.53) and poor aqueous solubility (0.01 mg/ml) [5]. Its absorption in gastrointestinal tract (GIT) occurs via proton-dependent active transport, which is partially shared with folic acid. MTX follows saturable (Michaelis–Menten) kinetics and thus its absorption becomes dose dependent in GIT [6,7]. Lower doses of MTX have higher bioavailability (42% for dose < 40 mg/m^2) compared with higher one (18% for dose > 40 mg/m^2). Bioequivalent dose of MTX was compared and mean intramuscular bioavailability was ≈ 76% compared with 33% (13 – 76%) via par oral route [8]. The maximum dose strength is 2.5 mg with narrow therapeutic window. Therapeutic and toxic plasma concentration of MTX are 0.005 and 0.01 µg/ml in low-dose therapy and 2.27 and 4.54, 0.23 and 0.45, and 0.02 and 0.04 µg/ml on high-dose therapy at 24, 48 and 72 h, respectively [9]. MTX shows high inter- and intra-patient variability in both serum and cerebrospinal fluid (CSF) [10]. Due to its erratic gastrointestinal absorption, it has been suggested that the dose ≥ 25 mg or above should be administered parenterally (intramuscular most often) [11]. Factors that influence its absorption include food, oral nonabsorbable antibiotics such as vancomycin, neomycin, bacitracin and more rapid transit through the GIT such as diarrhea (**Table 1**). However, slower transit in GIT such as constipation increases its absorption [11,12]. Plasma $t_{1/2}$ of MTX ranges from 2 to 10 h and it gets metabolized by intestinal bacteria into inactive metabolite 4-amino-4-deoxy-*N*-methylpteroic acid (DAMPA) and accounts for the loss of < 5% of its oral dose.

1.2 Pharmacodynamics

1.2.1 Basic pharmacology

MTX mainly acts by blocking the enzyme dihydrofolate reductase (DHFR) competitively thereby inhibit the production of thymidine (**Figure 1**). MTX specifically interferes with mitotic cell division. A clinical condition in which rapid cell division takes place includes neoplastic disease, autoimmune diseases and pregnancy. MTX inhibits syncytialization of cytotrophoblast thereby *in vitro* blocking the process of implantation rather than weakening the implantation site directly [13].

1.2.2 Adverse effect

The common adverse effects of MTX are nausea, vomiting, anemia, neutropenia, pulmonary fibrosis, diarrhea, dermatitis, bone marrow depression, mucositis, bruising, hepatitis, and so on. MTX shows dose- and duration-dependent teratogenicity. The fact had been justified by Savion *et al.* by studying the effect of MTX on Bax wild-type (WT) cell [14]. MTX is found to be iatrogenic too. Four cases of fatal MTX intoxication due to medical malpractices were reported in Tubingen Institute of Forensic Medicine, China, which showed the severe consequences of MTX overdose 10 mg (in two cases), 15 mg (in one case) and 20 mg

Article highlights.

- Methotrexate (MTX) is a weak dicarboxylic acid belonging to Biopharmaceutical Classification Systems class III, which acts by inhibiting dihydro folate reductase enzyme.
- Oral absorption of MTX is capacity limited and dose dependent. Thus controlled/sustained release formulation enhances its bioavailability over conventional formulations.
- Therapeutic drug monitoring (TDM) of MTX is prime requisite for treatment strategy due to its narrow therapeutic window and to minimize its side effects.
- Among various multiparticulate systems, liposomes and nanoparticles seem promising tool for site-specific delivery. However, they possess size-dependent property, stability and scale-up problems.
- The prodrug and drug conjugate approach suits best for targeted drug delivery and seems to be more specific than multiparticulate systems with least stability and scale-up issues. However, presence of specific functional group is mandatory for conjugation.
- Strong antileishmanial effect of MTX has been established due to its inhibitory action against dihydrofolate reductase.
- Clinical uses of MTX in cancer are well reported. However, MTX is not a versatile drug for all types of cancer and drug resistance is common.
- MTX is the preferred disease-modifying antirheumatic drug but not preferred among second-line antirheumatic drugs for patient with active rheumatoid arthritis (RA). There is less information available on tolerability, potency and safety on psoriatic arthritis treatment with MTX but abundant information is available on systemic manifestation of RA.
- A fixed-dose combination of MTX and misoprostol is best suited for medicolegal termination of pregnancy than single-dose regimen of MTX or misoprostol.

This box summarizes key points contained in the article.

(in one case) MTX daily instead of weekly leading to severe mucositis and death [15-17].

2. Methods of targeted drug delivery and controlled release systems of MTX

2.1 Controlled/Sustained release formulations

Variation in plasma $t_{1/2}$ of MTX requires repetitive administration within therapeutic range for its optimal bioactivity, as the cytotoxicity of MTX is purely dependent on mean resident time (MRT) in plasma [18]. An encapsulated lipid-based drug delivery system for cutaneous administration of MTX, developed by Bonetti *et al.*, showed increased plasma $t_{1/2}$ from 0.53 to 100 h (190 times) and lower C_{max} (120 times) than encapsulated drug with similar AUC. This extended-release formulation was 130 times more potent against L1210 leukemia cells without significant change in therapeutic index [19,20]. Similar formulation was developed for intracavitary administration and apparent $t_{1/2}$ was increased from 0.5 (unencapsulated MTX) to 39.6 h (encapsulated MTX) [21].

Another lipid-based formulation prepared by Chatelut *et al.* showed increased $t_{1/2}$ from 0.30 (unencapsulated) to 5.4 days (encapsulated) via intracisternal route [22]. The plasma drug level of MTX was maintained within therapeutic range with better antitumor activity for a longer period by chitosan microspheres [23]. A water-in-oil microemulsion-based controlled release (CR)-MTX emulsion suppresses tumor cell growth on multiple tumors and acts more predominantly by inducing apoptosis with longer duration of action [24]. MTX implants in external femoral condyle of rabbit showed prolonged release due to the phenomena of adsorption/desorption of MTX onto deficient apatite with calcium phosphate, which favors increased antirheumatic activity [25].

These controlled/sustained release (CR/SR) formulations have overcome the frequency of administration of MTX by providing controlled delivery to absorption sites but neither was able to achieve targeting approach nor minimized adverse effect significantly.

2.2 Effect of different routes of administration

The pharmacokinetics of MTX is purely dependent on route of administration. In order to compare the effect of route of administration of MTX level in CSF and blood in rats, it was found that the plasma levels from intranasal administration were significantly lower than that after intravenous (i.v.) administration. While concentration achieved in CSF after intranasal administration were significantly higher compared with i.v. administration [26]. The experiment favored intranasal route for CSF targeting compared with i.v. route. To access the efficacy of MTX, 33-week randomized double-blind, placebo-controlled crossover trial were designed. In this clinical trial, once-weekly 15 mg dose of MTX and placebo were compared for the treatment of chronic asthma, which would further reduce risk of exposure to steroids and its harmful effects [27].

Doddoli and his coworker administered MTX-Liposome (LSP-MTX) with a polymerized core (LSP) and free MTX into anesthetized rat by pulmonary instillation. They found that LSP-MTX was not oriented to spleen or kidney rather retained in alveolar tissues but free form of drug was cleared by reticuloendothelial system (RES). This liposomal approach can be used for pulmonary targeting of MTX and other anti-neoplastic drugs [28]. MTX was injected via transcutaneous puncture at the level of cisterna magna in a rodent model representing cognitive and neurotoxic effects. A significant reduction in folate level in CSF and serum was seen. However, homocysteine level in CSF was increased. The experiment supports for the establishment of an animal model for intrathecal delivery of MTX [29]. Cytotoxic study of MTX was performed in mouse bone marrow by Choudhary *et al.* Single intraperitoneal administration with three different doses (2, 10 and 20 mg/kg) were given. MTX was more clastogenic in male mice than the females. The intermediate dose (10 mg/kg) was found more effective than other doses [30].

Table 1. Drug interaction of methotrexate (MTX) with different drugs.

Drug	Effect	Mechanism	Ref.
NSAIDs, nephrotoxins, celecoxib β-lactams (penicillins, cephalosporins, carbapenems and monobactams), sulfonamides, phenylbutazone, probenecids, cisplatin, amphotericin-B, aminoglycosides, *Pueraria lobata* root decoction (Isoflavone)	Increases toxicity of MTX	Decreases renal Elimination of MTX	[11,127,16]
Phenylbutazone, salicylates probenecid, barbiturates, phenytoin, sulfamethoxazole, barbiturate, probenecid, tranquilizers, cefoperazone	Increases toxicity of MTX	Displacement from serum albumin binding	[11]
Nonabsorbable antibiotics, vancomycin, neomycin, nystatin, polymyxin-B	Decreases efficacy of MTX	Decreases absorption of MTX in GIT	[11]
Trimethoprim–sulfamethoxazole, ethanol, phenylbutazone	Increases toxicity of MTX	Pharmacodynamic enhancement of MTX toxicity	[11]
6-Mercaptopurine, other purine and pyrimidine	Increases efficacy of MTX	Synergistic mechanism	[17,18]
Dexamethasone	Increases hepatotoxicity of MTX	Reduces biliary elimination of MTX	[18]
Digoxin	Decreases digoxin effect	Reduces absorption	[19]

GIT: Gastrointestinal tract; NSAIDs: Nonsteroidal anti-inflammatory drugs; MTX: Methotrexate.

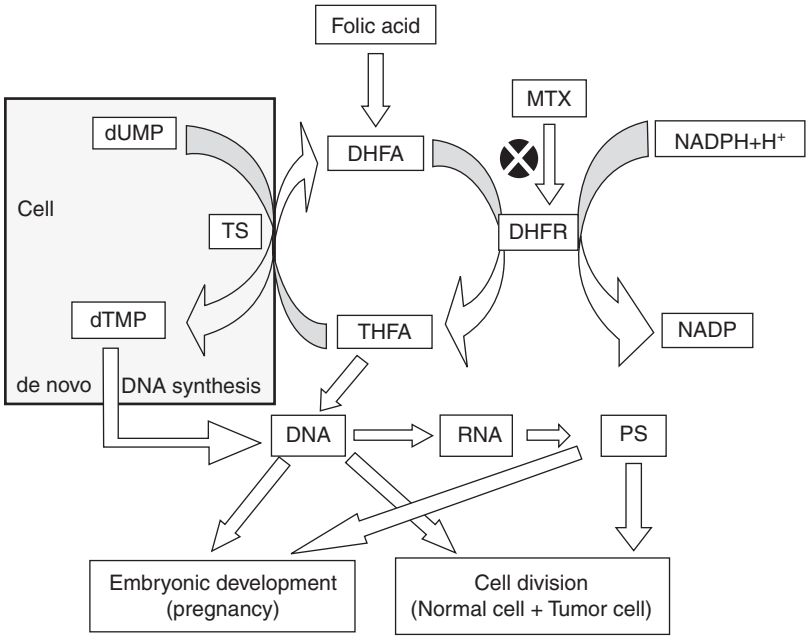


Figure 1. Schematic representation of mechanism of action of MTX.
DHFA: Dihydro folic acid; DHFR: Dihydro folate reductase; DNA: Deoxy ribonucleic acid; dTMP: Thymidylate; dUTP: Deoxyuridylate; MTX: Methotrexate; NADP: Nicotinamide adenine dinucleotide phosphate [13]; NADPH+H+: (reduced NADP); PS: Protein synthesis; RNA: Ribonucleic acid; THFA: Tetrahydro folic acid; TS: Thymidylate synthase.

It has been concluded from the above discussion that, first, some routes of administrations can be alternative approach for the targeting of MTX, but they may be selective and not optional for all kind of patients and drugs. Second, the alteration in route of administration has not yet overcome the problem of adverse effect at all.

2.3 Novel drug delivery systems

2.3.1 Microspheres

Microspheres are designed for prolonged release and improved pharmacological activity with reduced side effects. In this context, biodegradable hydrophilic gelatin microspheres of MTX in three different particle sizes (1 – 5, 5 – 10, 15 – 20 μm)

were prepared by polymer dispersion technique and cross-linked with glutaraldehyde. All had shown zero-order release of MTX for approximately 6 days in simulated gastric fluid and 5 – 8 days in simulated intestinal fluid. The *in vitro* drug release rate through these microspheres possessed inverse relation with particle size [31]. In an attempt to make magnetic microspheres of MTX (MM-MTX) by conjugation/complexation, MTX was PEGylated with PEG1500 to form a polyethylene glycol–MTX conjugate. (PEG-MTX) were subsequently added to a ferrous/ferric salt solution to give MM-MTX-I magnetic microspheres or PEG was added to ferrous/ferric ion salt solution coupled with MTX to form MM-MTX-II. *In vivo* releases from MM-MTX-I and MM-MTX-II in rat plasma were found approximately 97 (w/w) and 74% (w/w) over 24 h, respectively. The study showed that magnetic microspheres prepared by conjugation/complexation are stable and good candidate [32]. MTX-loaded poly lactide co-glycolide (PLGA) microspheres showed triphasic and molecular weight-dependent drug release. Its antitumor efficacy in Sarcoma-180 tumor-bearing mice showed increased MRT (18 ± 2.7 days) compared with plain subcutaneous injection (8 ± 0.7 days) along with CR [33].

Narayani *et al.* has studied MTX efficacy toward the solid tumor fibrosarcoma in Wistar rats. Injectable gelatin microspheres (10 – 20 µm) containing free MTX (GMFM) and covalently conjugated gelatin MTX using carbodiimide (GMCM-I, GMCM-II and GMCM-III) coupling were prepared. The MTX-loaded gelatin microspheres showed controlled release by gradual degradation of the spheres and concomitant diffusion of drug with enhanced antitumor activity compared with free MTX in the order of GMCM-I > GMCM-II > GMCM-III [34]. MTX-loaded immunomicrospheres, prepared by glutaraldehyde activation method, showed selective affinity for antigen-positive human leukemia cell line with less than 3% binding to nonspecific cells [35]. Radioactive microspheres of [H^3] MTX–human serum albumin (HSA) conjugates with particle size of 10.0 – 22.4 µm were injected into tail vein of mice. It had shown that total radioactivity in the lung increased immediately in a few minutes followed by gradual decrease after 3 – 4 weeks, apparently reaching up to a plateau phase. In contrast to lung, the radioactivity in liver, spleen and kidney increased slowly during the rapid depletion in radioactivity from lung. This suggested that small and large microspheres could be entrapped rapidly in lung through mechanical filtration owing to their large particle size and slowly redistributed to the liver, spleen, kidney and other parts of body [36].

MTX microspheres have overcome some pharmacokinetic and pharmacodynamic problems associated with conventional microspheres. However, these microspheres could be modified up to certain extent only by conjugation of some biocompatible polymers.

2.3.2 Liposomes

Being low-permeability drug, it is expected that pharmacokinetics of MTX could be improved by liposomes. Liposomes are microscopic vesicles made up of variety of lipid (mainly phospholipids and cholesterol) bilayers behaving as membrane surrounding the aqueous compartment inside, which makes it more accessible by biological membrane. A lot of research work has been highlighted toward application of liposomes in cancer treatment. A comparison has been made on liposome-mediated delivery of MTX-γ-aspartate to cell suspensions. It has been found that egg phosphatidyl glycerol, dioleoylphosphatidyl glycerol and dilaurylphosphatidyl glycerol containing liposomes inhibited cell growth more than dimyristoyl phosphatidyl glycerol and dipalmitoylphosphatidyl glycerol liposomes [37]. Similarly, negatively charged MTX-γ-aspartate liposomes were found more efficient for *in vitro* drug delivery with improved pharmacokinetics than neutral liposomes [38].

Kotsifaki *et al.* studied the targeting of MTX-loaded liposomes toward *Tetrahymena pyriformis* cells, which showed time- and concentration-dependent uptake of entrapped MTX. Surprisingly, the inhibitory effect of 4.5 µm MTX-loaded liposomes was equivalent to free MTX. It was found that liposomes prepared by phospholipids and/or gangliosides extracted from *T. pyriformis* cells had shown three times higher uptake than liposomes made up of commercial phospholipids [39].

The lipid bilayer in liposomes act as a good barrier to pass ionic and polar molecules, the permeability of which can be enhanced in their gel-to-liquid crystalline phase transition region. For this, thermosensitive liposomes have been prepared for cancer cell targeting. The transition temperature of 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) is 41.5°C just above the body temperature, which owes enhanced permeability and drug release at phase transition [40]. This principle was first used by Zhua *et al.* to produce thermosensitive magnetoliposome (TMs) by reverse-phase evaporation method in which MTX was encapsulated with DPPC and cholesterol to target skeletal muscle. Almost 60% of the drug at 37°C remained inside up to 24 h within TMs. However, more than 80% of MTX was released when temperature was raised from 37 to 41°C [41]. Immunoliposomes (ILs) prepared by the extrusion technique inhibited growth of A431 cells but had no effect on the growth of normal human fibroblastic cell lines. However, ILs prepared by reverse-phase evaporation technique were found less effective for the inhibition of A431 cell proliferation as compared with free drug [42]. The lymph node targeting and the pharmacokinetics of [^{3}H]MTX-loaded neutral large unilamellar vesicles (NLUV) and IL were compared with free [^{3}H]MTX after i.v. or i.m. injection to rats. It was found that the plasma radioactivity declined slowly after i.v. injection of the NLUV or IL compared with [^{3}H]MTX. This study has given idea about feasibility of lymph targeting of NLUV or IL than free [^{3}H] MTX approach [43].

In another study for the treatment of encephalomyelitis by MTX liposome, the feasibility of antigen targeting was evaluated. It was found that antigen-sensitive liposomal MTX inhibits proliferation of sensitized lymphocytes more than

nonsensitized lymphocytes with enhanced cytotoxic effect than nontargeted liposomes [44]. Similar approach was employed by Noe *et al.* in order to study the inhibition of cell proliferation with antibody-targeted liposomes. Liposomes containing MTX-γ dimyristoyl phosphatidyl ethanolamine (MTX-γ-DMPE) were covalently coupled with protein A. The coupling permitted specific association of liposomes *in vitro* with murine cells, which was previously incubated with protein A-binding monoclonal antibodies (mAb). It was observed that in the absence of antibody, MTX-DMPE liposomes did not result in greater binding to cells than liposomes made without MTX-γ-DMPE [45].

Singh *et al.* reported that the inhibitory effect of mAb (DAL K29) targeted MTX-containing liposomes as small unilamellar lipid vesicles (SULV) on human renal cancer. Tumor model was developed after intraperitoneal injection of 5×10^6 cells of the human kidney cancer line Caki-1. This study had showed that DAL K29 linked MTX-loaded SULV was more potent inhibitor ($p < 0.0005$) instead of free drug or mAb alone [46].

Topical delivery of drug is another key area for the application of liposomes. In order to study the effectiveness of dermal administration of MTX, deformable liposome were prepared using soybean lecithin phosphatidyl choline (PC) or hydrogenated lecithin (HPC) as phospholipid carrier and dipotassium glycyrrhizinate (KG) as surfactant in ratio of 2:1 and 4:1 (w/w) respectively. Almost four times higher permeation of MTX through pig skin was found from the liposomes containing KG compared with normal liposomes. No significant difference in permeation between PC-KG liposomes and HPC-KG liposomes were seen. On administration of deformable liposomes, 50% of the administered dose was found in skin. Study revealed that liposomes containing KG was effective in the treatment of psoriasis [47].

Structurally liposome resembles to the niosome. The only difference is that, instead of phospholipids in liposome, niosome consists of nonionic surfactants [48]. Niosome of MTX developed by Azmin *et al.*, using nonionic surfactant, cholesterol and dicetyl phosphate, has shown increased gastrointestinal absorption, liver and brain uptake owing to increased permeability when injected in mice via i.v. route. They also reported that biotransformation of MTX into 7-hydroxy MTX was also significantly reduced, which further resulted in increment of plasma $t_{1/2}$ of MTX [49].

It has been found that liposomes are more compatible to body tissues and could be efficient approach for site-specific delivery to enhance efficacy and minimize side effects. Bioavailability and pharmacokinetic problems could be resolved by lipid-based formulation. However, the particle size, stability and scale-up have always remained a great challenge to formulation scientist. The lipids/cholesterols present in liposome are prone to oxidation and rancidity. Lipid-soluble drugs will exhibit more entrapment efficiency compared with lipid-insoluble or least soluble drugs. Thus liposome does not suit for hydrophilic drugs.

2.3.3 Nanoparticles

2.3.3.1 Basic principle of cancer treatment and targeting with nanoparticles

The use of nanoparticles for cancer treatment is based on the fact that nanoparticles will release the active pharmacological moiety on tumor cells owing to enhanced permeability and retention effect.

Cancer treatment can also be done by active targeting of cancer cells by ligands present on the surface of nanoparticles. First, effectiveness of treatment is directly related with recognition of target cell and to kill the cancer cells without affecting healthy cells. Second, the nontumor cells are less permeable to nanoparticles limiting the drug distribution only to tumor cells [50]. This phenomenon is helpful in active and site-specific targeting of MTX-loaded nanoparticles.

2.3.3.2 Polymeric nanoparticles

The PEGylation of nanoparticles enhances circulation time and permeability of nanoparticles or liposomes [51]. Gao and Jiang studied the effects of particle size of polysorbate 80-coated polybutyl cyanoacrylate nanoparticles (70 – 345 nm) of MTX and its transport across blood–brain barrier (BBB) in rats. Coated nanoparticles exhibited higher concentration in brain than uncoated nanoparticles [52]. Yang *et al.* had prepared MTX-loaded nanoparticles (261.9 nm) of methoxy poly(ethylene glycol)-grafted-chitosan (mPEG-g-CS) conjugates by dialysis method via formaldehyde linkage. They proposed that continuous drug release (≥ 50%) in 48 h from mPEG-g-CS can be used as potential sustained release carrier of MTX [53].

Cationic bovine serum albumin (CBSA) and conjugated polyethylene glycol-poly (lactide) (PEG-PLA) (CBSA-NP) were tried as MTX carrier by Lu *et al.* These conjugates were prepared in different CBSA density, conjugated system and unconjugated system in order to assess brain delivery property and accelerated blood clearance using 6-coumarin as fluorescent probe in mice. The result across BBB showed that the increase in CBSA density of nanoparticles increases the BBB permeability-surface area but decreased blood AUC. The optimized conjugated system acquires higher concentration in brain by two to three times compared with unconjugated system [54]. A novel class of lipid nanoparticle, lipoprotein-mimicking biovectorized systems (LMBVs) for the delivery of MTX (70 – 76 nm), was prepared by Utreja *et al.* using microemulsion congealing technique. Palmitoyl polyethylene glycol 4000 (p-PEG 4000) anchored on LMBVs as apoprotein analog reduced the zeta potential and enhanced stability with zero-order release [55].

2.3.3.3 Solid lipid nanoparticles

Mulla *et al.* prepared MTX-loaded solid lipid nanoparticles (SLNs) using glyceryl monostearate, tripalmitin and tristearin as lipid carriers. The SLNs showed initially burst followed by controlled release of MTX [56]. Another MTX-loaded SLN was prepared by Ruckmani *et al.* using MTX, stearic acid and soya lecithin in the ratio of 1:4:1, 1:4:1.5 and 1:4:2 with an

average size of 270 nm. The $t_{1/2}$ and MRT were increased than plain MTX solution. Life span of Ehrlich Ascites Carcinoma (EAC)-bearing mice was increased too [57]. In a study of topical treatment of psoriasis, MTX-loaded SLNs were incorporated in gel using Compritol 888, cetyl alcohol, stearic acid as lipid carrier and Tween 80 as surfactant. The entrapment efficiency was found to be 52.16%. *In vitro* skin deposition studies showed significantly higher deposition of SLN through skin [58]. Topical delivery of nanogel of MTX consisting of copolymerized *N*-isopropylacrylamide (NIPAM) and butylacrylate (BA) polymer in the presence of Na_2CO_3 was studied by Singka *et al.* Porcine epidermal membranes mounted in Franz diffusion cells *ex vivo* were used. MTX-loaded nanogel when applied showed 33 and 57% reduction in PGE_2 level in absence and presence of Na_2CO_3, respectively [59].

Nanoparticles of MTX have proven as useful commodity in bioavailability and pharmacokinetic enhancement with minimum or no side effects. The rancidity problem seen in liposome can be overcome by nanoparticles. SLNs are promising agents for site-specific delivery and are more effective compared with polymeric nanoparticles. Furthermore, its commercialization and stability problems are the challenges remained to be resolved. The stability problems are mostly seen in multiparticulate systems compared with conventional formulations.

2.3.4 Miscellaneous multiparticulate systems

Resealed erythrocytes are gathering wider interest as targeted drug delivery carriers for cancer treatment and gene delivery. In the context of carrier-mediated delivery of MTX for liver targeting, Mishra *et al.* conjugated MTX with *N*-hydroxysuccinimide ester of biotin (NHS-biotin) and loaded on erythrocyte. The macrophage uptake and phagocytic index of these modified erythrocytes were found to be two times more compared with nonbiotinylated drug-loaded cells and higher stability assessed by glycerol lysis in rat. Their finding supports that erythrocyte could be used as a potential novel carrier for liver targeting with excellent stability and biocompatibility [60].

Trotta *et al.* studied the effect of counter ions (monooctyl phosphate, monodecyl phosphate, etc.) on mouse skin for permeation of MTX from water in oil microemulsion containing lecithin (surfactant) and water–propylene glycol (internal phase) at different pH. Permeation was increased due to lipophilization of MTX as a consequence of ion pair formation. It could be concluded that the topical delivery of MTX greatly enhanced once formulating the drug as ion pair with sodium dodecyl sulfate and dioctyl sulfosuccinate [61].

Lymphatic targeting is a good approach to eliminate first-pass metabolism. MTX-loaded chylomicron mimicking carrier emulsion consisting of Compritol 888 ATO (CA, lipid core) and soya lecithin (PC, stabilizer) were prepared by Paliwal *et al.* The mean particle size and drug entrapment efficiency were 160.3 ± 10.2 nm and 72.8 ± 6.5%, respectively The *in vivo* studies were carried out in albino rats and performance were estimated by collecting blood and lymph. The formulation showed increased concentration in lymph [62]. Self-aggregates of MTX-loaded poly(2-hydroxyethyl aspartamide) copolymers were prepared by Kang *et al.* It was found that the amphiphilic nature of the drug induces initial burst release in the buffer medium. Irrespective to the amount of octadecyl chains, PEGylation with MTX suppressed the initial burst release [63].

MTX-encapsulated dendrimers composed of polyether-co-polyester (PEPE) with varying degree of orientation were prepared. It was observed that increase in the number of branches with PEGylation and size of internal voids increases encapsulation efficiency. Whereas loading efficiency gets reduced in the absence of aromatic rings that may act as branching units to trap the drug moiety. Increase in the number of branching decreases this initial burst release while absence of aromatic rings in dendritic structure promotes rapid release [64]. Latallo *et al.* prepared PAMAM dendrimers in which the MTX and folic acid were attached. They injected these conjugates in immunodeficient mice bearing human KB tumors that overexpress the folic acid receptor. Folate-conjugated nanoparticles were concentrated in the tumor and liver tissue over 4 days after administration compared with nontargeted polymer. Prior i.v. injection of free folic acid could attenuate tumor tissue localization of the PAMAM dendrimers [65].

2.3.5 Prodrug and drug conjugates

2.3.5.1 Mechanism of action and drug release

MTX has been used as the active component of macromolecular prodrug. Presence of carbonyl and amino group at terminal position of MTX makes it good candidate for conjugation (**Box 1**). MTX is found stable within lysosomal acidic environment suggesting lysosomotropic mechanism for activation of drug [66]. Fitzpatrick and Garnett studied mechanism of action of MTX-HSA-mAb conjugates and found lysosomal digestion of conjugate for its release at target-specific drug [67]. A list of prodrugs and conjugates of MTX with their importance has been summarized in **Table 2**.

2.3.5.2. Pharmacological actions and methods of drug targeting

Several literatures are available on conjugation and targeting of MTX with peptides [68-70], antibodies [71-74], enzymes [75,76], target carrier system [77], serum albumin [78], radiolabeled peptides [79,80]. In a study, MTX was conjugated with gelatin using 1-ethyl-3-(diaminopropyl) carbodiimide HCl (EDC). The effect of different variables (pH, amount of conjugating agent on release characteristics, composition and stability of conjugate was studied by Kosasih *et al.* MTX released from the conjugates at different temperatures such as 25, 37 and 50°C, in isotonic solution, had no effect of gelatin molecular weight with approximately linear release and first-order release rate constant [81]. Growth inhibition study in different molar ratio (MR) of MTX:gelatin conjugate ranged from 1:1 to 27:1. It was found that higher MR conjugates produced

Table 2. Summary of prodrug and drug conjugates of methotrexate (MTX) and their importance.

Coupling agent	Spacer/via	Pharmacological effect	Inference	Ref.
Polypeptides containing Poly (L-LYS)	Carbodiimide reaction	Reduced *in vitro* cytotoxicity	Poly (L-LYS) does not suits to effective chemotherapy	[68]
Gelatin	1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide HCl	Prolonged plasma $t_{1/2}$	May improve bioavailability and pharmacological activity	[70]
α-Alanine, arginine phenylalanine, aspartate	–	Addition of carboxypeptidase A increases cytotoxicity	Potentially useful for tumor targeting	[69]
Proline	–	Enhanced cytotoxicity to resistant tumor	Good approach to overcome cancer resistance	[71]
Fibrinogen	*N*-hydroxy-succinimide ester	Higher antitumor activity in mice with P388 leukemia	Therapeutic utility of fibrinogen–MTX conjugate established	[75]
anti-791T mAb-791T/36	HSA	Selectively cytotoxic for cells bearing 791T/36-antigen with improved cytotoxicity to resistant tumor	Favors targeting And overcome resistance	[72]
Lysine	Para-aminobenzyloxy carbonyl (PABC)	Selective binding toward HSA	Favors site-specific delivery	[76]
TNP-labeled poly (D-lysine)	Disulfide or triglycine	Selective binding toward Fc receptor-bearing cells	Favors site-specific delivery	[77]
murine mAb (aMM 46)	HSA	Selectively cytotoxic to antigen (MM46)	Favors site-specific delivery	[78]
immunoglobulin (nIg)	HSA	More cytotoxic to MM46 than MM48	Favors site-specific delivery	[74]
LBSA and BSA	Carbodiimide reaction	[^{3}H]MTX-LBSA was higher (81%) than [^{3}H]MTX-BSA (56%) in Kupffer cells	[^{3}H]MTX-LBSA favors site-specific delivery	[79]
IgM, anti-SSEA-1 and IgM, MOPC 104E antigens	–	Higher concentration of Immunoconjugate detected in Kupffer cells and hepatocytes	Favors site-specific delivery	[73]
Bombesin (BN) labeled with ^{99m}Tc	–	High affinity toward breast and prostate cancer cell	Favors site-specific delivery	[80]

BN: Bombesin; BSA: Bovine serum albumin; HSA: Human serum albumin; LBSA: Lactosaminated bovine serum albumin; PABC: Para-aminobenzyloxy carbonyl; TNP: Trinitrophenyl.

less growth inhibition, greater stability and less gelatin degradation in conjugate by lysosomal enzyme Cathepsin B compared with low MR conjugates [82].

In another study to improve antineoplastic selectivity by prodrug approach, MTX was acylated with various α-amino acids at 2-amino group position, which are predicted to be hydrolyzed by aminopeptidase enzyme localized/targeted to tumor cells. Prodrug conjugation of MTX with L-pyroglutamyl compound, L-leucyl, L-valyl, L-isoleucyl, D-alany and L-pyroglutamyl derivatives decomposed slowly when incubated in phosphate buffer (pH 7.3) and readily hydrolyzed to free MTX by aminopeptidase M. These derivatives were considerably lesser cytotoxic than MTX, except L-leucyl derivative (cytotoxic). These results indicate that 2-L-leucyl-MTX failed as a prodrug since it gets activated prematurely by serum enzymes [83].

A second-generation prodrug in MTX α-peptide series was designed for activation of MTX by CP-mAb conjugates, MTX-α-phenylalanine (MTX-Phe). Prodrug was prepared by reaction of the *p*-nitrophenyl ester of 4-amino-4-deoxy-10-methylpteroic acid with L-glutamyl-α-L-phenylalanine. Conversion of MTX-Phe to MTX by bovine pancreas carboxypeptidase A (CP-A) was 250-fold faster than corresponding reaction involving MTX-α-alanine. Thus earlier one was the best MTX peptide substrate for the enzyme. The amount of CP-A required to make MTX-Phe equitoxic with MTX, on human lung adenocarcinoma cells (UCLA-P3) *in vitro* was 10 times lower [84].

In another study for selective targeting of MTX on human ovarian teratocarcinoma cells (CRL-1572), prodrug MTX-phenylalanine (MTX-Phe) was conjugated with mAb-4E3 and bovine CP-A conjugate (4E3 + CPA). *In vitro* cytotoxic assay of CRL-1572 cells showed that prodrug alone was nontoxic but conjugated prodrug enhances its pharmacological activity (ID_{50} of 70 ng ml^{-1}) in considerable amount in a selective manner [85].

Pignatello *et al.* studied inhibition of lipophilic MTX–lipoamino acid conjugates on bovine liver DHFR against MTX-sensitive human lymphoblastoid (CCRF-CEM) cells and an MTX-resistant subline (CEM/MTX). Experiment revealed that CEM/MTX cells were very less susceptible to inhibition than CCRF-CEM cells by α- or α,γ-substituted lipoamino acid conjugates. However, both cell lines were almost equally sensitive to the MTX-γ conjugates. This experiment proved that lipophilic MTX conjugates may be good lead compounds in the drug development for the treatment of some MTX-resistant tumors [86].

Li and Kwon studied micelle-like structure of MTX conjugate. They attached MTX on poly(ethylene oxide)-block-poly (2-hydroxyethyl aspartamide) (PEO-b-PHAA), based on the assumption that attachment of MTX on PEO-b-PHAA through an ester bond, the amphiphilic conjugate would gradually release MTX due to unfavorable hydrolysis of nonpolar core. ^{1}H-NMR analysis showed that PEO-b-PHAA-MTX conjugates were self-assembled to form supramolecular structure where MTX was residing in a site with highly restricted mobility. They concluded that PEO-b-PHAA-MTX conjugate micelles may help to improve the biodistribution of MTX and help to overcome drug resistance [87].

Kaasgaarda *et al.* synthesized two lipophilic analog of MTX in which C_{15}-alkyl chain attached to γ-carboxylic acid in one of the analog and in other analog additional benzyl group attached to α-carboxylic acid. They studied the cytotoxicity of prodrug against KATO III and HT-29 human colon cancer cells were independent of alkylation of γ-alkylated compound. However, addition of benzyl group at α-carboxyl group rendered the compound to be nontoxic. It was concluded that alkylated MTX analogs were available only for cancer cell uptake, independent of liposome hydrolysis and resulting in a tumor-specific release of MTX derivative [88].

The above described strategies employed single enzyme or cell targeting. In another approach, Santos *et al.* targeted MTX by aminopteroyl-based hydroxamate derivatives to target both matrix metalloproteinase (MMP) and DHFR for antitumor activity. This new hydroxamate derivative of MTX was found to be effective inhibitor of MMPs and DHFR within micromolar and nanomolar concentration, respectively, and showed strong antiproliferative activity against A549 cells (non-small cell lung carcinoma), PPC-1 and Tsu-Prl prostate cancer cell lines [89].

Mctavish *et al.* found that the insulin-like growth factor (IGF) receptor is overexpressed on many types of cancer cells and could be used to target MTX to tumor cells. MTX IGF-1 conjugate was found more effective than free MTX at 6.25 times lower dose *in vivo* [90]. Subr *et al.* conjugated MTX with hexamethylene diamine (HMDA) by modification of α-carboxylic [HMDA-MTX(I)] and γ-carboxylic [HMDA-MTX(II)] group of glutamic acid. The two conjugates, HMDA-MTX(I) and HMDA-MTX(II), were coupled with poly[*N*-(2-hydroxypropyl) methacrylamide] via oligopeptide (-GlyGly-, GlyLeuGly- and Gly-DL-PheLeuGly)- as spacers. The result showed that release rate of HMDA-MTX(I) and HMDA-MTX(II) from the polymer was dependent on the detailed structure of prodrug, which could be controlled by structural modification of oligopeptide spacer [91]. An MTX conjugate prepared by nonselective amide formation had shown the release of only 5% of free drug after 48 h in the lysosomes [92].

Many antibody-directed enzyme prodrug therapy (ADEPT) have been employed for cancer targeting of antineoplastic agent including MTX. It could be done by two-step process. In step-I, the prodrug targeted enzyme–antibody (EA conjugate that activates prodrug) conjugate is administered. In step-II, antibody–drug (prodrug conjugate) conjugate is administered. EA conjugate administered previously in step-I gets oriented toward tumor cells (**Figure 2**). In step-II when prodrug conjugate is administered, it is selectively cleaved by EA conjugate at tumor site and thus active drug is released at tumor site [92,93].

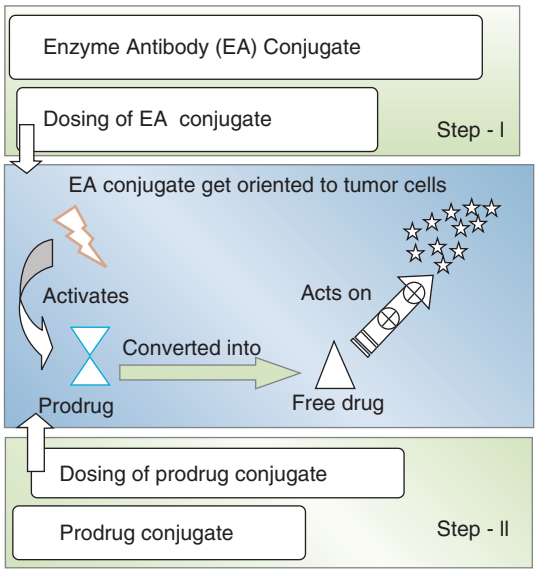


Figure 2. Schematic representation of mechanism of action of antibody-directed enzyme prodrug therapy.

Based on this principle, two analogs of MTX, MTX-α-3-cyclobutylphenylalanine and MTX-α-3-cyclopentyltyrosine (prodrug conjugate), and enzyme human carboxypeptidase A1 (hCPA1) and T268G] were conjugated with ING (an antibody that binds to tumor antigen, Ep-Cam) to make ING-1:hCPA1-T268G (EA conjugate). The results of EA conjugate showed excellent activation of prodrug to kill HT-29 cells as efficiently as MTX itself at targeted site [94].

To reduce the toxic effects of folic acid in host cell, MTX was coupled in two ways: directly to anti-Ly-2.1 antibody (approximately 10 molecules of MTX per antibody molecule) and indirectly to HSA (approximately 24 molecules of MTX per antibody molecule). It was found that direct coupling leads to loss of potency of MTX (30-fold less potent than free MTX). Whereas indirect coupling of MTX-HSA conjugates were three to five times less potent compared with free MTX. However, both conjugates were more specific. It has also been found that increase in the number of drugs per conjugate led to increased cancer cytotoxicity [67,95].

The combined properties of hyaluronic acid (HA) and MTX were used for rationale treatment of osteoarthritis. HA alone reduces pain but do not fully control inflammation. Oral MTX has anti-inflammatory activities, but side effect is severe. In osteoarthritic treatment, Homma *et al.* used HA, a synovial fluid having a lubricating effect for conjugation. HA–MTX conjugate produced a significant reduction of knee swelling in antigen-induced arthritis in rat. Whereas free MTX or HA or a mixture of both showed no significant effects [96].

Conjugation and prodrug approach have been found to be most suitable for selective delivery and recognition within special tissues or cells. First, the development of prodrug and conjugates is exclusively attributed to availability of specific functional group or conjugating agent. Thus prodrug and conjugation systems did not need much attention regarding particle size and stability-related issues and thus they possess least size stability problem, which remained major challenge among multiparticulate systems. Second, scaling-up of prodrug-based formulations is easier than others. However, presence of complementary functional group is the mandatory requirement for conjugation between drug and conjugating agents. All the targeted drug delivery systems of MTX are limited only up to research laboratory and have not been practiced for clinical applications and treatment.

3. Antileishmanial effect of MTX

MTX shows strong antileishmanial effect due to its inhibitory action against DHFR. Chakraborty *et al.* showed that MTX coupled to mannosyl BSA strongly inhibits the growth of leishmania parasites inside macrophages by binding specifically to the mannose receptor on macrophages (100 times more active than free MTX). These mannosylated coupled MTX are internalized and degraded in lysosomes and subsequently release the active drug to act on the parasites [97,98]. MTX conjugated with mannosylated albumin, which targeted to macrophages via scavenger receptors, has been shown to inhibit leishmaniasis [99,100]. This approach could also be used for targeting of other antileishmanial drugs.

4. Clinical aspects of MTX

4.1 MTX in cancer treatment

4.1.1 Specific pharmacology

MTX acts by blocking DHFR enzyme, which converts dihydro folic acid (DHFA) to tetrahydro folic acid (THFA) (**Figure 1**). This inhibition of THFA causes failure of protein synthesis (central dogma). Ultimately, inhibition of protein synthesis delays or inhibits cell division. Thus the growth of dividing cell gets inhibited.

4.1.2 Clinical indication

MTX is one of the oldest, most commonly used and highly efficacious antineoplastic drugs (**Figure 3B**). Urothelial carcinoma is a chemosensitive malignancy. Phase II data of clinical trial showed 29% response. Treatment with cisplatin and MTX is considered as superior combination for urothelial cancer [101]. In a clinical study conducted on 105 women subjects with low-risk gestational trophoblastic neoplasia, the efficacy of single-dose MTX was accessed. An i.v. bolus of 100 mg/m^2 of MTX followed by in fusion of 200 mg/m^2 over 12 h was administered. The remission rate was found to be 84 and 44% in women acquiring single-dose regiment. In a woman failing single-dose MTX regimen required further doses of MTX, the hCG level prior to treatment was found to be higher [102]. In another study, MTX alone was given to 337 patients of nonmetastatic gestational

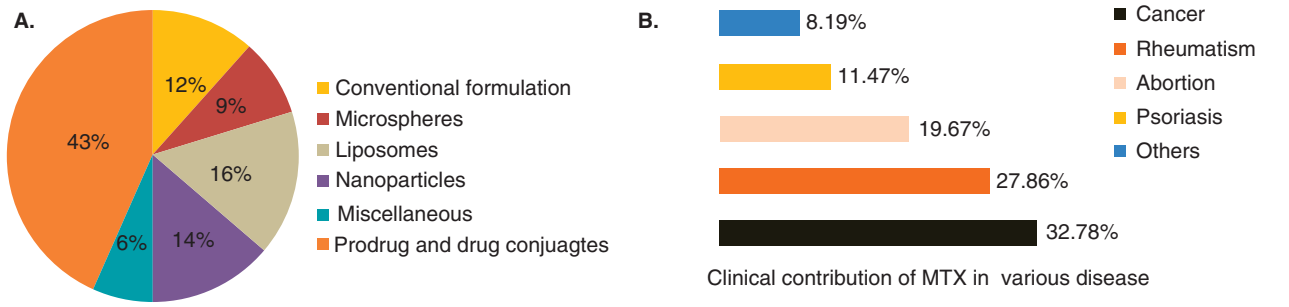


Figure 3. (A) Comparative involvement of R&D of methotrexate (MTX) in different drug delivery systems. (B) Clinical contribution of MTX in various diseases.

trophoblastic tumors. Of that, 253 patients (75.0%) were treated initially with MTX (0.4 mg/kg/day alone intravenously) for 5 days, repeatedly every 14th day. All 337 showed complete cure among which resistance developed in 27 [103].

Kohne conducted Phase-II clinical trial and studied that the cytotoxic behavior of MTX and *N*-phosphonacetyl-L-aspartate acid (PALA) modulates the cytotoxic effects of 5-fluorouracil (5-FU). To evaluate the toxicity and efficacy of PALA/MTX and 5-FU, study has been conducted with effective dose scheduling of PALA/MTX/5-FU to treat colorectal cancer and hyperglycemia, which is a common side effect in diabetic patients requiring therapeutic drug monitoring (TDM) [103,104]. Hematological, gastrointestinal and renal toxicity assessment in 24 h showed that TDM of MTX can reduce the toxicity of MTX. Combination of MTX with ifosfamide, etoposide and cytarabine has showed increased toxicity [105]. Results of a randomized Phase II study, comparison of effect of docetaxel with MTX in patients suffered from recurrent head and neck cancer showed that docetaxel and MTX had an overall survival of 3.7 versus 3.9 months and time to progression of 1.97 versus 1.5 months, respectively [106].

Clinical use of MTX in cancer treatment is well established. However, MTX is not a versatile drug for all types of cancer and development of its resistance is common. MTX is apparently curative in choriocarcinoma and highly effective in maintaining remission in children with acute leukemia. However, MTX is not good for inducing remission. A fixed-dose combination is better opted.

4.2 MTX and RA at a glance

RA is an autoimmune disease comprising autoimmune and inflammatory responses [107]. Zintzaras *et al.* has proved that due to long-term efficacy in clinical practice, currently MTX is the preferred disease-modifying antirheumatic drug but not first among second-line antirheumatic drug for treatment of patients with active RA [108,109]. However, many patients did not experience remission and have continued signs and symptoms of active disease while receiving a maximally tolerated dose [109].

4.2.1 *Specific pharmacology*

MTX is widely accepted as the gold standard in RA treatment. It potentially acts via antiproliferative, anti-inflammatory and/or immunosuppression [110]. The anti-inflammatory activity of MTX is mediated by suppression of activation and adhesion molecule rather than lymphocyte apoptosis [111]. Though full effect and mechanisms are still uncertain, the chemotherapy of RA using MTX is anti-inflammatory than immunosuppressive. The anti-inflammatory action of MTX in RA mainly acts by inhibition of mediators of inflammation such as interleukins (IL-1, IL-6, tumor necrosis factor 1 (TNF) activity and other inflammatory cytokines). MTX also inhibits or decreases aminoimidazo-carboxamide transformylase action and increases adenosine release at its low dose [107].

4.2.2 *Clinical use and indications*

A guideline was prepared for the use of MTX in clinical treatment of RA, which was based on its doses and routes of administration, investigations, folate supplementation, optimal management strategy, complete blood cell count, serum transaminase levels, serum creatinine clearance, special consideration chest radiograph, serological tests for the hepatitis viruses B and C prior to starting MTX therapy [112]. Alefacept (intramuscularly) in combination with MTX was administered to assess the efficacy and safety in patients suffered with psoriatic arthritis (PsA). After 12 weeks, observation of PsA patient showed that combination was quite effective in treatment of both psoriasis and PsA [113].

In order to assess the clinical benefits of long-term MTX therapy for RA, 39 among 124 patients showed improvements. However, nausea, stomatitis, hair loss, rash, pulmonary reactions, elevated liver enzymes, hematologic abnormalities and hepatic fibrosis were the adverse reactions associated with permanent discontinuation of MTX. It was concluded that the main factor leading to discontinuation of MTX therapy was adverse reaction [114]. To study the effect of folate supplement during MTX therapy, out of 200 RA patients taking low-dose MTX, 42 patients have shown elevation in mean cell volume (MCV) whereas normal MCV were seen in remaining 158 patients. In continuation of this, 198 patients

were supplemented with oral folic acid and there was no significant adverse effect to MTX therapy in folate-supplemented patients except heart burn, which occurred only in the patients with high MCV [115].

The efficacy, side effects and risk factors of low-dose MTX in RA as second-line agent were studied by Schnabel. There is less information available on tolerability, potency and safety on psoriatic arthritis treatment with MTX but abundant information available on systemic manifestation of RA, spondyloarthritis and collagen vascular diseases. Treatment of severe hemocytopenia and pneumonitis (life threatening) is the major treatment concern with MTX rather than bothering about its hepatotoxicity [116]. Pulmonary 'hypersensitivity reactions' are one of the toxicity concerns in early RA (1 – 7.6%) and do not seem to be favorable but may be used in conjugation with other drugs for the treatment of early RA [117]. It has been reported that MTX should not be used directly if nonsteroidal anti-inflammatory drugs fail, as first among second-line antirheumatic. Its pulmonary toxicity may lead to systemic fungal infections and unexplained significant weight loss [111].

4.3 Medical termination of pregnancy using MTX and misoprostol: what is the link?

4.3.1 *Specific pharmacology*

The mechanism by which MTX acts as an abortive agent is described in **Figure 1**. The embryo in uterus is highly totipotent. The totipotent nature of cells requires high degree of protein synthesis for proper growth and differentiations. The inhibition of DNA along with inhibition of protein synthesis interferes embryonic cell division thus leads to abortion or termination of pregnancy. MTX is used in conjunction with misoprostol (an analog of prostaglandin E_1). Misoprostol causes the softening of cervix and the contraction of uterus by interacting with prostaglandin receptors resulting in expulsion of the uterine contents [118].

4.3.2 *Clinical use and indications*

The usual dose employed is 50 mg/m^2 MTX and 800 µg misoprostol for medical abortion. Creinin *et al.* studied the safety and pharmacokinetic parameters of MTX given as either 50 mg/m^2 (group I) or 60 mg/m^2 (group II) in 20 women for early abortion at randomized controlled trial, at ≤ 49 days of gestation. MTX plasma level was monitored for 24 h interval for 7 days followed by 800 µg misoprostol administered vaginally on the 7th day of MTX administration. In group I 90% abortion occurred compared with 100% in group II. Plasma T_{max} was found to be 1 – 2 h in both group and disappeared in 48 h in group I and 72 h in group II along with higher plasma concentration and AUC [119].

In a multicenteric Cohort study, MTX, 50 mg/m^2 intramuscularly, was administered followed by misoprostol 800 µg vaginally (5 – 6 days later, repeated in 1 or 2 days in the absence of abortion) in 25 pregnant adolescents (less than 18 years old) up to 49 days of gestation. The result showed 96% abortion, 92% immediate abortion and 17% felt negative experience with single failure of abortion [120]. A multicenteric trial was employed by Creinin *et al.* with similar dose regimen among 300 women seeking abortion. Of that, 263 women had shown complete and immediate abortion within 24 h following administration of misoprostol for early 49 days of gestation. Vaginal bleeding lasted up to 14 ± 7 days and 11 ± 9 days as immediate success and delayed success in the patients respectively [121]. Similar approach was studied by Carbonell *et al.* with similar results [122].

The similar study of Ozeren to assess the fixed-dose combination and single-dose regimen of MTX and misoprostol, with similar dose regimen out of 108 subjects (9 weeks of gestation or less) showed complete 69% abortion in group receiving 50 mg/m^2 MTX only, 58% receiving 800 µg vaginal misoprostol only and 89% abortion in group receiving combination. The study suggested the fixed-dose combination for effective abortion [123]. Many similar dose regimens of MTX and misoprostol were employed clinically for efficacy in medical abortion. All of these showed better with combined dose regimen [124]. Stika *et al.* had assessed effect of single-dose regimen of MTX in the treatment of ectopic pregnancy. Fifty patients with mean β-hCG level 1896.4 ± 2399 mIU/ml were given single-dose MTX according to the protocol of Stovall *et al.* The result showed only 32.64% abortion with average success rate of 78% in one to three doses of MTX with lowering of β-hCG level and 22% failed for termination and required surgery [125].

To compare the efficacy of tamoxifen along with MTX followed by misoprostol, 198 women with less than 7 weeks of gestation were given either 40 mg of tamoxifen followed by 800 µg of misoprostol (2 – 3 days later) vaginally or 50 mg/m^2 of MTX followed by the same dose of misoprostol (5 – 7 days later) in Phase I. In Phase II, 20 mg tamoxifen and rest similar dose regimen were used. Higher success rate was found in MTX group (93.0%) compared with the tamoxifen group (85.7% requiring a surgical aspiration, with less side effect compared with Phase II) in Phase I. In MTX group, 90.9% of abortion compared with 84.7% in the tamoxifen group (p 5 0.20) were found in Phase II [126].

5. Conclusion

MTX, a DHFR inhibitor, is a promising agent for clinical treatment of various diseases such as cancer, RA, psoriasis, abortion and other autoimmune diseases. Safety margin of MTX is narrow, thus treatment requires TDM to minimize life-threatening adverse effects. Oral absorption of MTX is capacity limited. Design of its controlled release formulation in a novel drug delivery form could prove efficacious in avoiding the plasma drug level fluctuations and also improve patient compliance by reducing the dosing frequency for better bioavailability than immediate-release formulation by per oral route. Therapeutic efficacy of several novel drug delivery approaches such as microspheres, nanoparticles, solid lipid

Table 3. Different marketed products of methotrexate (MTX).

Dosage form	Brand name	Company	Country	*Dose*
Tablet	IMUTREX®	Cipla	India	2.5 mg/7.5 mg/10 mg
Tablet	METHOREX®	Zydus (G.Rem)	India	2.5 mg
Tablet	MEXATE ®	Cadila HC	India	2.5 mg/7.5 mg
Tablet	NEOTREXATE®	GSK	India	2.5 mg
Tablet	ONCOTREX®	Sun	India	2.5 mg/7.5 mg/10 mg
Tablet	REMTREX®	Alkem	India	2.5 mg
Tablet	Meisusheng®	Hospira	China	2.5 mg
Tablet	Methotrexat Ebewe®	Ferron/Ebewe	Indonesia	2.5 mg
Tablet	Emthexate®	Pharmachemie	Malaysia	2.5 mg
Tablet	Methotrexate	Orion Pharma	Finland	10 mg
Tablet	Maxtrex	Pharmacia Ltd.	New Jersey, United Kingdom	2.5 and 10 mg
Tablet	METHOBLASTIN	Pfizer Ltd	United Kingdom	2.5 and 10 mg
Tablet/Injection	Trexan	Orion Pharma	Finland	2 and 10 mg
Tablet	Methotrexate	Sandoz	Denmark	5 mg
Tablet	Lantarel	Wyeth Pharmaceuticals Division	Germany	2.5, 7.5 and 10 mg
Tablet	Emthexate	ASTA Medica Australasia Pty Ltd.	Netherlands, Belgium, Norway, Portugal, Spain, Greece	50 and 500 mg
Tablet	DBL MTX®	Hospira	Singapore	2.5 mg
Capsule	Rheumatrex®	Wyeth	Japan	2 mg
Capsule	Methotrexate®	Mylan Seiyaku	Japan	2 mg
Capsule	Methotrexate®	Towa pharma	Japan	2 mg
Injection	IMUTREX®	Cipla	India	15 mg x 1 mL
Injection	ONCOTREX®	Sun	India	10 mg x 1 mL
Injection	MTX®	GeneraMedix Inc	USA	2 ml, 10 ml, 40 ml
Injection	MTX	Bolai Pharm	China	100 mg × 1 mL
Injection	MTX	Pfizer	China	50 mg/2 ml × 1 mL
Injectable	ZEXATE	FLAKON Pharmaceutical Companie	Turkey	15 and 50 mg
Injection	Farmitrexat (Methotrexat-Dinatrium)	Pharmacia & Upjohn	Germany	5,48 mg/21,92 mg/ 54,8 mg/548 mg/ 1096 mg/5480 mg
Injection	Ledertrexate	Wyeth Pharmaceuticals Division	Netherlands, Belgium, France, Portugal, Finland	5 and 25 mg
Injection	Emthexate	ASTA Medica Australasia Pty Ltd.	Sweden, Spain	2.5, 5, 25 and 100 mg
Vial	BIOTREXATE®	Biochem	India	5 mg x 1's, 50 mg
Vial	METHOREX®	Zydus (G.Rem)	India	5 mg/25 mg/100 mg x 1 mL
Vial	Meisusheng®	Hospira	China	500 mg x 20 mL
Vial	Methotrexat Ebewe®	Ferron/Ebewe	Indonesia	5 mg/1 mL
Vial	Enbrel®	Wyeth	Malaysia	25 mg
Vial	DBL MTX®	Hospira	Singapore	50 mg/2 mL
Vial	Methotrexate	Hospira UK Ltd.	England	50 mg/2 ml, 250 mg/ 10 ml and 500 mg/20 ml
Ampoule	FOLITRAX®	IPCA	India	7.5 mg/15 mg x 1 mL
Ampoule	Methotrexat Ebewe®	Ferron/Ebewe	Indonesia	5 mg/1 mL
Gel	REXTOP®	Systopic	India	1% w/w x 10 g/20 g

nanoparticles, liposomes, prodrug and drug conjugates have been studied and proved challenging advantageous tool to minimize the adverse effects and maximize its therapeutic outcome with site-specific drug delivery. The prodrug and drug conjugate approach suits best for targeting and more biocompatible than multiparticulate systems and could be commercialized. Clinical trials for treatment of RA and cancer have showed its broad-spectrum activity. Induction of abortion along with misoprostol has been studied and showed fruitful results when present in fixed-dose combination than alone.

6. Expert opinion

The facts and figures described in this review give sufficient evidence about the clinical efficacy of MTX along with its

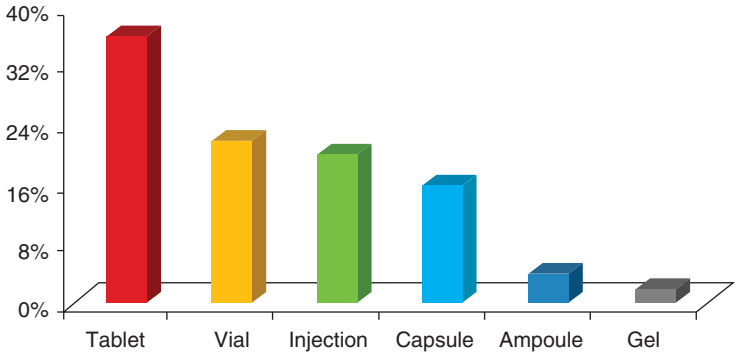


Figure 4. Available dosage forms of methotrexate (MTX) in various national and international markets.

superiority related to its safety profile over its wide use as conventional dosage forms. The attention should be paid on its clinical application, which must be a site-specific treatment rather than exposure to the whole body. The said aim could efficaciously be achieved by various multiparticulate systems such as nanoparticles, solid lipid nanoparticles and liposomes for targeting of various forms of malignancy and RA. Though multiparticulate and novel approaches such as prodrug and drug conjugates have proved successful platform to meet this ultimate goal, the application and research are limited to research laboratory rather than industrial and clinical applications (**Figure 3A**). Major limitations are stability, scaling-up (problem associated with pharmaceutical commercialization) and lack of knowledge by clinicians or physicians. In spite of huge R&D and advantage of novel drug delivery systems, pharmaceutical market is still dominated by conventional formulations that are cheap and easy to manufacture (**Table 3**, **Figure 4**).

Fixed-dose combination of MTX with other drugs should also be formulated and clinically tested for clinical trial in treatment of cancer, abortion, RA, psoriasis, clinical treatment of leishmaniasis and other autoimmune diseases. The ongoing research and clinical trials of drug delivery systems of MTX require more attention to make more approachable and affordable treatment to the patients. The research is of no more importance until it is able to be commercialized and consumed by patients. Therefore, there is a need to design and develop various simple, cost-effective, efficacious dosage forms and also to upgrade existing manufacturing skills at commercial point of view, which can open alternative horizons and new avenues in the field of drug delivery.

The remarkable research approaches have been highlighted in this review, which require special attention of clinicians who are practicing over only its conventional dosage forms. Another problem associated to manufacturing technology and scaling-up of its novel drug delivery systems requires more attention of pharmaceutical experts to refine such technologies that could be utilized in the scaling-up of such novel formulation in a more economical way. Apart from above discussion, health, environmental and socioeconomic hazards are the other unavoidable risks in pharmaceutical practice of nanotechnology. Therefore, these unstudied areas provide upcoming opportunity for researchers, clinicians and health specialists, especially those experts related to cancer and rheumatoid disease, to work together and explore the treatment benefits of this drug to the patients.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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